

the Examiner alleges that the limitation "dehydrating to a water content of at least 28 g/100 g dwt" in claims 24 and 32 was supported by neither the generic disclosure, or the specific examples. Applicants traverse. Contrary to the Examiner's allegation, Table 4 of Example 4 (page 12) discloses that a water content of 28 g/100 dwt or higher would be necessary for an acceptable percentage rate of regenerating explants after freezing the explants in liquid nitrogen. Thus, the rejection has been overcome and should be withdrawn.

On
but
what?
plant?

Claims 14-33 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Specifically, the Examiner objects to claims 14, 24 and 31 on the ground that it is not clear whether "zygotic embryo" is excluded from the claimed methods. Applicants traverse and respectfully bring the Examiner's attention again to the fact that "zygotic embryo" is explicitly categorized in the specification (lines 25-26, page 1) as an explant "in developmental late stages," which is further excluded in the generic disclosure from the present invention.

The Examiner also states that it is uncertain what is the difference between a "primary regenerating tissue" in claim 14, and a "primary explant" as in claims 24 and 31. Applicants respectfully traverse. The specification discloses that a plant derived tissue namely "primary explant" may be cultivated in an induction medium to generate a "primary regenerating tissue." (lines 21-29, page 3). In other words, the difference between a "primary explant" and a "primary regenerating tissue" is that the latter is generated after an induction step, while the former is not. Thus, the rejection has been overcome.

Claims 14, 24 and 31 were further objected to for the recitation of an "induction medium" and a "regeneration medium." The Examiner states that the two media contain the same sucrose concentration and there is no apparent difference between them. In response, Applicants have replaced the phrase "regeneration medium" in claims 24 and 31 with the phrase "induction medium" to avoid any potential confusion. Thus, the Examiner's objection has been obviated.

Claims 27-30 were objected to for lack of antecedent basis for "the plant tissue." The Examiner also objects to the use of "dwt" in claims 24 and 32. In response, Applicants have amended claim 24 and 32 to correct the minor informalities.

Claims 14, 16-19, 31 and 33 were rejected under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 6,143,563 to Peterson ("Peterson"). Applicants traverse.

Peterson discloses a method for cryopreserving plant callus without the use of cryoprotectants or programmable freezers. In Example 1 of Peterson, the callus is obtained by incubating embryos at 28 °C in the dark to form calli after 11 days. In contrast, the present invention relates to a process for the cryo-preservation of a primary explant, which again explicit excludes "zygotic and somatic embryos, meristems, etc." as discussed above. Since embryos as used in Peterson are already considered by the present invention as being in a developmental late stage and thus excluded from the present invention, the callus obtained from an embryo after incubating it at a high temperature would certainly also be excluded from the present invention. Therefore, the present invention is not anticipated by the Peterson. The Examiner's rejection has been overcome and should be withdrawn.

OK
Peterson
Hatanaka
Callus
not
(meristems)

Claims 14-20, 22, 31 and 33 were rejected under 35 U.S.C. 102(b) as being anticipated by Pence. Applicants traverse. Pence discloses a process for the cryopreservation of zygotic embryos. Although the zygotic embryos in Pence can be derived from *Theobroma cacao*, zygotic embryos are excluded from the scope of the present invention as previously discussed. Therefore, Pence does not anticipate Applicants' invention and therefore, this rejection should be withdrawn.

Lastly, claims 14-33 were rejected under 35 U.S.C. 103(a) as being unpatentable over Peterson taken with Pence, U.S. Patent No. 5,943,821 to Ducas *et al.* ("Ducas") and U.S. Patent No. 5,922,929 to Zimmerman *et al.* ("Zimmerman"). Applicants traverse.

Peterson and Pence have already been discussed above and neither discloses a process involving a primary explant as the present invention concerns. Ducas and Zimmerman are replied upon by the Examiner to demonstrate that plant species such as *Coffea canephora* and *Daucus carota* is capable of forming a callus culture. However, all of the references concern only the cryopreservation of callus culture. None of the references cited by the Examiner disclose or suggest a primary explant should be used in the cryo-preservation process. In contrast, the present invention relates to the cryopreservation of a primary explant which explicitly excludes callus as being in a developmental late stage. Thus, the rejection should be withdrawn.

The Examiner has indicated that the amendment of the claims to remove the "but not a somatic embryo" would result in the re-imposition of the obviousness rejections over the Hatanaka *et al.*, Lecouteux *et al.* and Abdebnour-Esquivel *et al.* references. This procedure would be incorrect, since those references disclose somatic or zygotic embryos which are not encompassed by the claims. As explained above, the "primary explant" language

distinguishes the invention from these references. These references are no more relevant to the present claims than those that were cited in this action. Accordingly, the claims should not be rejected over these references for the same reasons presented above.

Furthermore, applicants discovered and disclose in the specification that by using a primary explant instead of a somatic or zygotic embryo as taught by the prior art, one is able to unexpectedly increase the rate of successful regeneration while lowering the percentage of somaclonal variation inherent in cryo-preservation of plants. Also, a wide variety of plants may be cryopreserved using the process utilizing primary explants as disclosed in the present invention. Nothing in the references cited by the Examiner teach or suggest these advantages that result from the presently claimed invention.

Because none of the references together or alone teach or suggest the use of a primary explant in a process of cryo-preservation, nor the unexpected advantages associated with using a primary explant instead of somatic or zygotic embryos, Applicants' invention is not made obvious.

In view of the foregoing, it is believed that the entire application is now in condition for allowance, early notice of which would be appreciated. Should any issues remain, a personal or telephonic interview is respectfully requested to discuss the same in order to expedite the allowance of all the claims in this application.

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Respectfully submitted,



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APPENDIX A - MARKED UP VERSION OF AMENDED CLAIMS

14. (Amended) A process for the cryo-preservation of a primary explant comprising the step of cryofreezing the primary explant, wherein the primary explant comprises a plant tissue that has been subjected to an induction medium for a time sufficient to induce a primary regenerating tissue[, but not a somatic embryo].

24. (Amended) A process for the cryo-preservation of a primary explant comprising the steps of:

incubating a plant [planting] tissue in an induction[a regeneration] medium for a time sufficient to induce a primary explant[, but not a somatic embryo];

dehydrating the primary explant to a water content of at least 28 g/100g dry weight [dwt];

prefreezing the primary explant to a temperature between -20°C and -40°C; and
cryofreezing the primary explant.

31. (Amended) A process for the cryo-preservation of a primary explant comprising the steps of:

incubating a planting tissue in an induction[a regeneration] medium for a time sufficient to induce a primary explant[, but not a somatic embryo]; and
cryofreezing the primary explant.

32. (Amended) The process of claim 31, further comprising the step of dehydrating the primary explant to a water content of at least 28 g/100g dry weight [dwt].

APPENDIX B - LISTING OF PENDING CLAIMS

14. (Amended) A process for the cryo-preservation of a primary explant comprising the step of cryofreezing the primary explant, wherein the primary explant comprises a plant tissue that has been subjected to an induction medium for a time sufficient to induce a primary regenerating tissue.
15. The process of claim 14, further comprising a two step incubation of the primary explant, wherein the primary explant is first incubated in a medium containing 0.4 M sucrose followed by incubating the primary explant in a medium containing 1 M sucrose.
16. The process of claim 14, further comprising the step of dehydrating the primary explant prior to cryofreezing.
17. The process of claim 16, wherein the dehydration step involves placing the primary explant in an air current of a laminar flow cabinet, in a stream of compressed air, or in an airtight container together with silica gel or various over-saturated salt solutions to control the relative humidity.
18. The process of claim 14, further comprising the step of pre-freezing the primary explant prior to cryofreezing.
19. The process of claim 18, wherein the pre-freezing temperature is between -20°C and -40°C.
20. The process of claim 14, wherein the plant tissue utilized is derived from a cocoa, coffee, or carrot plant.
21. The process of claim 20, wherein the plant tissue utilized is derived from *Coffea canephora* or *Coffea arabica*.
22. The process of claim 20, wherein the plant tissue utilized is derived from *Theobroma cacao*.

23. The process of claim 20, wherein the plant tissue utilized is derived from *Daucus carota*.
24. (Amended) A process for the cryo-preservation of a primary explant comprising the steps of:
- incubating a plant [planting] tissue in an induction medium for a time sufficient to induce a primary explant;
 - dehydrating the primary explant to a water content of at least 28 g/100g dry weight;
 - prefreezing the primary explant to a temperature between -20°C and -40°C; and
 - cryofreezing the primary explant.
25. The process of claim 24, further comprising a two step incubation of the primary explant, wherein the primary explant is first incubated in a medium containing 0.4 M sucrose followed by incubating the primary explant in a medium containing 1 M sucrose.
26. The process of claim 24, wherein the dehydration step involves placing the primary explant in an air current of a laminar flow cabinet, in a stream of compressed air, or in an airtight container together with silica gel or various over-saturated salt solutions to control the relative humidity.
27. The process of claim 24, wherein the plant tissue utilized is derived from a cocoa, coffee, or carrot plant.
28. The process of claim 24, wherein the plant tissue utilized is derived from *Coffea canephora* or *Coffea arabica*.
29. The process of claim 24, wherein the plant tissue utilized is derived from *Theobroma cacao*.
30. The process of claim 24, wherein the plant tissue utilized is derived from *Daucus carota*.

31. (Amended) A process for the cryo-preservation of a primary explant comprising the steps of:

incubating a planting tissue in an induction medium for a time sufficient to induce a primary explant; and

cryofreezing the primary explant.

32. (Amended) The process of claim 31, further comprising the step of dehydrating the primary explant to a water content of at least 28 g/100g dry weight.

33. The process of claim 31, further comprising the step of prefreezing the primary explant to a temperature between -20°C and -40°C.